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(54) **Antifungal compositions.**

(57) Antifungal compositions of aculeacin A and specific fatty acids, such as oleic, linoleic, linolenic, lauric, palmitoleic, arachidonic and palmitelaidic surprisingly increase the intrinsic activity of aculeacin A against fungi, such as *Candida albicans*.

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ANTIFUNGAL COMPOSITIONS

The instant invention relates to antifungal compositions containing aculeacin A and an amount of fatty acid selected from the group consisting of oleic acid, linoleic acid, linolenic acid, lauric acid, palmitoleic acid, arachidonic acid, and palmitelaidic acid sufficient to potentiate the bioactivity of aculeacin A. These compositions surprisingly increase the intrinsic activity of aculeacin A against fungi, such as Candida albicans strains B 311, BC 759 and B 3153A. These strains are maintained in the Smith Kline & French Laboratories culture collection.

Illustrative of the instant invention are antifungal compositions wherein between 5 and 3500 units by weight of oleic acid are combined with one unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.039 µg. Particular antifungal compositions are those where between 10 and 350 units by weight of oleic acid and one unit of aculeacin A are combined. Specific antifungal compositions of the instant invention contain between 50 and 3500 units by weight of oleic acid for each unit of aculeacin A, when at least 0.4 µg of aculeacin A is employed.

Similarly, antifungal compositions of the instant invention contain between 5 and 360 units by weight of linoleic acid for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.4 µg. Particularly, antifungal compositions containing between 40 and 360

units by weight of linoleic acid and one unit of aculeacin A are described when at least 0.625 ug of aculeacin A is employed.

Further, antifungal compositions of the instant invention contain between 5 and 180 units by weight of linolenic acid and one unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.4 µg. Specifically, between 40 and 180 units by weight of linolenic acid and one unit of aculeacin A are combined to afford an antifungal composition when at least 0.625 µg of aculeacin A is employed.

Additional illustrations of the antifungal compositions of the instant invention are those compositions wherein between 250 and 1000 units by weight of arachidonic acid and one unit of aculeacin A are combined and wherein between 500 and 2000 units by weight of palmitelaidic acid and one unit of aculeacin A are combined when at least 1.0 µg of aculeacin is employed.

Aculeacin A is a known antifungal antibiotic disclosed and described in the Journal of Antibiotics 30(4), pp. 297-313 (1977).

The antifungal activity of aculeacin A and the compositions of aculeacin A and fatty acids was measured by disc diffusion. Potentiation of antifungal activity was examined by three different methods employing: (1) a single concentration of aculeacin A and a single concentration of known fatty acids; (2) various concentrations of aculeacin A with a single concentration of individual fatty acids; and (3) various concentrations of fatty acids with a single concentration of aculeacin A. The antifungal activities of aculeacin A and the fatty acids were separately checked as controls. The fatty acids, per se, employed in the compositions of this invention do not exhibit antifungal activity at the concentrations tested.

The effects of a number of fatty acids on the antifungal activity of aculeacin A were determined by

utilizing seeded plates of Candida albicans B 311 in yeast nitrogen base (YNB) agar (Difco) with glucose as the carbon source. A composition containing one unit by weight of aculeacin A and 5 units of fatty acid or the sodium salt thereof was spotted on the seeded plates and the size of the zone of inhibition after 16-18 hours incubation at 28-37°C was measured. The results of the above test are shown in Table I.

TABLE I

Fatty acid	Zone Size (mm)
none (control)	15
Oleic Acid (sodium salt)	32
Palmitoleic Acid	26
Lauric Acid	22
Linoleic Acid	23
Linolenic Acid	22
Stearic Acid	15
Palmitic Acid	15
Myristic Acid	16
Decanoic Acid	17
Caprylic Acid	16

Aculeacin A and fatty acid compositions of Table I which afford an increase of 3 millimeters over aculeacin A demonstrated sufficient increase in the intrinsic activity of aculeacin A to be deemed synergistic.

Additional fatty acids were tested to determine if they increased the intrinsic activity of aculeacin A against C. albicans B 311 utilizing seeded plates of YNB agar (Difco) with lysine as the carbon source and seeded plates of Sabouarud dextrose agar (Difco). Compositions containg one µg of aculeacin A and various amounts of the fatty acid was spotted on the seeded plates and the size of the zone of inhibition after 16-18 hours incubation at 37°C was measured. The results of the above tests are shown, as well as a control, are shown in Table II.

TABLE II

Aculeacin A (µg)	Fatty Acid (µg)	Zone Size (mm) YNB agar	Zone Size (mm) Sabouarud Agar
1	None (control)	15	13
1	None (control)	16	15
	Erucic Acid		
0 (control)	1000	0	0
1	1000	15	Trace
0 (control)	500	0	0
1	500	17	17
1	500	15	17
0 (control)	250	0	0
	Vaccinic Acid		
0 (control)	2000	0	0
1	2000	17	26
0 (control)	1000	0	0
1	1000	17	25
0 (control)	500	0	0
1	500	17	25
	Linoelaidic Acid		
0 (control)	1000	0	0
1	1000	16	24*
0 (control)	500	0	0
1	500	17	20*
0 (control)	250	0	0
1	250	26	20*
	Arachidonic Acid		
0 (control)	1000	0	0
1	1000	26	20*
0 (control)	500	0	0
1	500	20	20*
0 (control)	250	0	0
1	250	20	20*

TABLE II (continued)

		Homo-γ-Linoleic Acid		
0	(control)	200	0	0
1		200	17*	15
0	(control)	100	0	0
1		100	15*	15
0	(control)	50	0	0
1		50	15*	15
		Palmitelaidic Acid		
0	(control)	2000	0	0
1		2000	22	25
0	(control)	1000	0	0
1		1000	20	32
0	(control)	500	0	0
1		500	20	30
		α-Hydroxy Lauric Acid		
0	(control)	1000	Trace	10 (hazy)
1		1000	25	20
0	(control)	500	Trace	10 (hazy)
1		500	24	15
0	(control)	250	Trace	Trace
1	(control)	250	20	15
		γ-Linoleic Acid		
0	(control)	2000	0	0
1		2000	22	25
0	(control)	1000	0	0
1		1000	15	24
0	(control)	500	0	0
1		500	14	20

TABLE II (continued)

Behenic Acid				
0	(control)	1000	0	0
1		1000	15	20
0	(control)	500	0	0
1		500	14	14
0	(control)	250	0	0
1		250	14	15
Eladic Acid				
0	(control)	1000	0	0
1		1000	13	13
0	(control)	500	0	0
1		500	13	15
0	(control)	250	0	0
1		250	13	18
Oleic Acid Sodium Salt				
0	(control)	1000	0	0
1		1000	35	26
0	(control)	500	0	0
1		500	35	26
0	(control)	250	0	0
1		250	35	28

*irregular zone of inhibition

Aculeacin A and fatty acid compositions of Table II which afforded an increase of 3 millimeters over Aculeacin A in both the YNB agar and the Sabouarud dextrose agar demonstrated a sufficient increase in the intrinsic activity of aculeacin A to be deemed synergistic. In addition to the above described compositions, the composition of 250 units by weight linoelaidic acid to one unit aculeacin A and the

composition of 2000 units by weight γ-linoleic acid to one unit aculeacin A exhibit a surprising increase in the intrinsic activity of aculeacin A.

The antifungal effects of compositions containing a single amount of oleic acid as the sodium salt while varying the amounts of aculeacin A were determined by utilizing seeded plates of Candida albicans B 311 in Sabouarud dextrose agar and measuring the zone of inhibition after 16-18 hours incubation at 28-37°C. The results of the above test, as well as a control, are shown in Table III.

TABLE III

Aculeacin A (ug)	Oleic Acid (ug)	Zone Size (mm)
2.5	0	19
1.25	0	14-16
1.25	12.5	37
0.625	0	10
0.625	12.5	35
0.312	0	trace
0.312	12.5	35
0.156	12.5	32
0.078	12.5	29
0.039	12.5	28
0.020	12.5	trace
0.000	12.5	0

The antifungal effects of compositions containing a single amount of aculeacin A while varying the amounts of oleic acid as the sodium salt were determined by utilizing seeded plates of Candida albicans strains B 311, BC 759 and B 3153A and measuring the zone of inhibition after 16-18 hours at 28-37°C. This test was also performed with linoleic acid and linolenic acid. The results of these tests are shown in Table IV-a, IV-b and IV-c.

TABLE IV-a

Aculeacin A (µg)	Oleic Acid (µg)	Zone Size (mm)		
		B 311	BC 759	B 3153-A
0.40	0	10	13	12
0.40	5	13	13	13
0.40	10	14	15	15
0.40	20	15	18	18
0.40	40	17	18	17
0.40	78	20	20	18
0.40	156	26	24	25
0.40	312	30	28	25
0.40	625	32	29	28
0.40	1250	35	32	29

TABLE IV-b

Aculeacin A (µg)	Linoleic Acid (µg)	Zone Size (mm)
		B 311
0.625	225	19
0	225	Trace
0.625	112.5	21
0	112.5	0
0.625	56.2	22
0	56.2	0
0.625	28.1	22
0	28.1	0
0.625	0	16

TABLE IV-c

Aculeacin A (µg)	Linolenic Acid (µg)	Zone Size (mm)
		B 311
0.625	112.5	19
0	112.5	0
0.625	56.2	19
0	56.2	0
0.625	28.1	20
0	28.1	0
0.625	0	16

The seeded plates of Candida albicans strains B 311, BC 759, and B 3153A were prepared by inoculating 50 ml of trypticase soy broth (BBL) with one ml of the preserved frozen culture and incubating the inoculum at 37°C for 7 hours on a rotary shaker. Two milliliters of this culture were used to inoculate one liter of Sabouarud dextrose agar (Difco) at 50°C and 15 milliliters of the resultant medium poured into 150 mm petri-dishes.

The antifungal activity of the compositions of the instant invention, as exemplified by the composition containing Aculeacin A and oleic acid as shown below, was demonstrated by topical treatment of C. albicans infections of mice. Clinical isolates of C. albicans were grown in Sabouarud dextrose agar and subcultured into mouse serum and incubated at 35°C. Female mice (CF-1, 4 to 6 weeks old) were shaved on the back ($2cm^2$ area). The shaved area was cleaned with 70% alcohol and scrubbed with a wire brush. This area was then painted with the C. albicans in mouse serum as noted above (10^5-10^6 CFU/ml.) and after drying this area was covered with sterile gauze and the infection was allowed to develop over two days. After the development of the infection, the mice were treated with aculeacin A/oleic acid composition, aculeacin A and Lotrimin^R (Clotrimazole-Schering) for 5 days. The minimum inhibitory concentration (MIC) for aculeacin A for each clinical isolate of C. albicans was determined and that amount was employed in each experiment. Commercially available Lotrimin^R cream was utilized in each experiment. As the results below demonstrate, the aculeacin A/oleic acid composition afforded a significant reduction in the infection (comparable to Lotrimin^R) over aculeacin A, itself, or the control. Oleic acid was tested separately and did not inhibit the infection. Note that bacterial contamination was ruled out by microscopic examination.

Experiment Number	Untreated Control	Lotrimin^{R}	Aculeacin A (ug)	Aculeacin A/Oleic Acid (ug/ug)
I	4 3	0 0	1 1 (0.8)	0 0 (0.5/100)
II	2 2	1 1	0 1 (1.5)	0 0 (0.9/100)
III	3 3	0 0	1 1 (5.0)	0 0 (1.5/100)
IV	3 3	0 0	2 2 (0.5)	1 0 (0.5/100)
V	3 3	0 0	0 0 (10.5)	0 0 (5.0/100)
VI	3 3	0 0	1 1 (5.0)	1 1 (5.0/100)

where 4 - the most severe infection - zone of inoculation highly erythrematous with culture mainly consisting of C. albicans.

3 - severe infection with some healing

2 - less severe infection

1 - very little infection

0 - no infection.

In view of the above described antifungal activity, antifungal amounts of the compositions of the instant invention may be employed as the active ingredients in pharmaceutically acceptable vehicles, such as solid, semi-solid or liquid carriers, to combat fungus growth. The instant compositions in pharmaceutical form may be applied to the area to be treated by conventional methods, such as, dusting, spraying, brushing, smearing, impregnating or other suitable means. The instant compositions may also be employed in agricultural forms to treat fungus growth in cultivated fields.

Claims:

1. A composition comprising an antifungal effective amount of aculeacin A and an amount of fatty acid selected from the group consisting of oleic acid, linoleic acid, linolenic acid, lauric acid, palmitoleic acid, arachidonic acid and palmitelaidic sufficient to potentiate the bioactivity of aculeacin A.

2. An antifungal composition of claim 1 which is effective against Candida albicans infection.

3. An antifungal composition of claim 1 wherein the fatty acid is oleic acid.

4. An antifungal composition of claim 3 wherein the amount of oleic acid is between 5 and 3500 units by weight for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.039 µg.

5. An antifungal composition of claim 4 wherein the amount of oleic acid is between 10 and 350 units by weight for each unit of aculeacin A.

6. An antifungal composition of claim 4 wherein the amount of oleic acid is between 50 and 3500 units by weight for each unit of aculeacin A, when at least 0.4 µg of aculeacin A is employed.

7. An antifungal composition of claim 1 wherein the fatty acid is linoleic acid.

8. An antifungal composition of claim 7 wherein the amount of linoleic acid is between 5 and 360 units by weight for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.4 µg.

9. An antifungal composition of claim 8 wherein the amount of linoleic acid is between 40 and 360 units by weight for each unit of aculeacin A when at least 0.625 µg of aculeacin A is employed.

10. An antifungal composition of claim 1 wherein the fatty acid is linolenic acid.

11. An antifungal composition of claim 10 wherein the amount of linolenic acid is between 5 and 180 units by weight for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.4 µg.

12. An antifungal composition of claim 11 wherein the amount of linolenic acid is between 40 and 180 units by weight for each unit of aculeacin A when at least 0.625 µg of aculeacin A is employed.

13. An antifungal composition of claim 1 wherein the fatty acid is arachidonic acid.

14. An antifungal composition of claim 12 wherein the amount of arachidonic acid is between 250 and 1000 units by weight for each unit of aculeacin A when at least 1.0 µg of aculeacin A is employed.

15. An antifungal composition of claim 1 wherein the fatty acid is palmitelaidic acid.

16. An antifungal composition of claim 15 wherein the amount of palmitelaidic acid is between 500 and 2000 units by weight for each unit of aculeacin A when at least 1.0 µg of aculeacin is employed.

17. A composition comprising an antifungal effective amount of aculeacin A and an amount of a fatty acid selected from the group consisting of lineolaidic acid and γ-linoleic acid sufficient to potentiate the bioactivity of aculeacin A wherein the amount of lineolaidic acid is 250 units by weight to one unit of aculeacin A and wherein the amount of γ-linoleic acid is 2000 units by weight to one unit of aculeacin A.

Claim (Austria)

1. A process for the preparation of an antifungal composition comprising an antifungal effective amount of aculeacin A and an amount of fatty acid selected from the group consisting of oleic acid, linoleic acid, linolenic acid, lauric acid, palmitoleic acid, arachidonic and palmitelaidic acid sufficient to potentiate the bioactivity of aculeacin A; which comprises mixing the required amounts of aculeacin A and fatty acid.